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Research and Development

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Project Summary

Investigation of Effects of Prolonged Inhalation of Nickel-Enriched Fly Ash in Syrian Golden Hamsters

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Groups of 102 Male Syrian Golden hamsters were chronically exposed to ~70 μg/l respirable Nickel Enriched Fly Ash aerosol (high NEFA group), ~17 μg/l (low NEFA group), or ~70 μg/l FA 6 hrs/day, 5 days/week for up to 20 months. An identical control group received sham exposures. The NEFA particles of respirable size contained approximately 6% nickel, compared to about 0.3% for FA. Five hamsters/group were sacrificed after 4, 8, 12, or 16 months of exposure. An additional 5 hamsters/group were withdrawn from exposure at the same intervals for lifelong observations.

Exposures to NEFA had no significant effect on the apparent well-being, body weight and life span of the animals although heavy deposits of NEFA in the lungs were demonstrated. The lung weights of the high NEFA- and of the FA-exposed animals, however, were significantly ($P<0.01$) higher than those of low NEFA controls. There was no significant difference between the mean body weights of the high NEFA group and the FA group. The mean lung volumes were significantly ($P<0.01$) larger for the high NEFA group and the FA group than for the low NEFA group and the controls.

There was a 100% incidence of dust deposition, referred to in this report as anthracosis, in the lungs of exposed hamsters. Incidence and severity of interstitial reaction and bronchiolization were significantly higher in the dust-exposed groups than in the sham-exposed controls. The severity of anthracosis, interstitial reaction and bronchiolization was significantly lower ($P<0.01$) in the low NEFA group than in the high NEFA and FA groups. This dose-effect relationship reflects the two different dose groups, namely low NEFA versus high NEFA and FA. While two malignant primary thorax tumors were found in two hamsters of the high NEFA group, no statistically significant carcinogenesis was observed. Of the exposure-related changes, only anthracosis decreased as a function of recovery time.

Comparison of pulmonary nickel burdens after 20 months of exposure with the aerosol concentration suggests that the pulmonary clearance rate was slower in the high NEFA exposure group than in the low NEFA exposure group.

The results of this study lead to the conclusion that the addition of nickel to fly ash under these experimental conditions did not significantly

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(P<0.05) enhance the pathogenicity (including carcinogenicity) of fly ash in this animal model.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Clinical, epidemiological and laboratory studies implicate nickel and certain of its compounds as an environmental health hazard. Exposure of the general population to nickel occurs mainly through the inhalation of fly ash. Fly ash is a combustion product of coal and fuel oil. It is discharged into the atmosphere at an estimated rate of several million metric tons per year in the United States. This quantity of fly ash contains several thousand tons of nickel. Because of its known toxicity and presence in fly ash, it was desirable to investigate whether nickel in fly ash is an important etiological factor. This was accomplished by enriching regular fly ash with nickel and exposing hamsters to nickel-enriched fly ash (NEFA) or to regular fly ash (FA) and comparing the findings to those in sham-exposed controls. NEFA was prepared by mixing nickel acetate into pulverized coal before combustion in a special fossil fuel furnace and collecting the fly ash generated in the process.

A pulmonary deposition, translocation and clearance study with neutron-activated fly ash to determine the fate of the inhaled fly ash in the animals, and an Ames assay to determine mutagenicity complemented the inhalation study.

Results and Conclusions

Exposure to NEFA had no significant effect on the apparent well-being, body weight and life span of the animals, although heavy deposits of NEFA in the lungs were demonstrated. The lung weights of the high NEFA- and of the FA-exposed animals, however, were significantly ($P<0.01$) higher than those of the low NEFA group and the controls. There was no significant difference between the MBWs of the high NEFA group and the FA group. The mean lung volumes were significantly ($P < 0.01$) larger for the high NEFA group and the FA group than for the low NEFA group and controls.

There was a 100% incidence of dust deposition, referred to in this report as anthracosis, in the lungs of exposed hamsters. Incidence and severity of interstitial reaction and bronchiolization were significantly higher in the dust-exposed groups than in the sham-exposed controls. The severity of anthracosis, interstitial reaction and bronchiolization was significantly lower ($P<0.01$) in the low NEFA group than in the high NEFA and FA groups. This dose-effect relationship reflects the two different dose groups, namely low NEFA versus high NEFA and FA. While two malignant primary thorax tumors were found in two hamsters of the high NEFA group, no statistically significant carcinogenesis was observed. Of the exposure-related changes, only anthracosis decreased as a function of recovery time.

Comparison of pulmonary nickel burdens after 20 months of exposure with the aerosol concentration suggests that the pulmonary clearance rate was slower in the high NEFA exposure group than in the low NEFA exposure group.

The results of this study lead to the conclusion that the addition of nickel to fly ash under our experimental conditions did not significantly ($P<0.05$) enhance the pathogenicity (including carcinogenicity) of fly ash in our animal model.

The fly ash burden estimates from the pulmonary deposition, translocation and clearance experiment, as determined by the radionuclides ^{46}Sc and ^{59}Fe, are in good agreement for the majority of samples analyzed. Such close agreement indicates fly ash *particulate* levels in the lungs, carcass, head, pelt, GI tract, and feces rather than leached radionuclides. Relative to the ^{46}Sc and the ^{59}Fe-based estimates, fly ash deposition estimates obtained with the isotope ^{60}Co were appreciably lower for the lungs and appreciably higher for one or more sacrifice times for carcass, liver, head, pelt and urine samples. This indicates that ^{60}Co (and thus the element cobalt) was selectively leached from fly ash deposited in the deep lung, translocated to other sites, and excreted in the urine.

An estimated average of 63 μg fly ash, or 2 to 3% of the inhaled fly ash, was initially retained in the respiratory tract. The estimated biological half-times of the fly ash were 2.6 and 34.5 days, probably for the airways and for the deep lung, respectively. After 99 days, the mean lung burden had decreased to about 10% of its initial value. Estimated near-complete clearance of fly ash from the lung would have been achieved approximately 200 days postexposure.

In all Ames assays the responses were negative. Thus, NEFA and FA did not appear to be mutagenic in the *standard* Ames assay. However, this does not preclude the possibility of demonstrating mutagenic activity by altering the standard Ames test in some way or by use of different extraction procedures to remove potential mutagens from the surface of NEFA and FA.

Materials and Methods

Based on the results of acute and subacute toxicity studies described in this report, groups of 102 male Syrian golden hamsters were chronically exposed to ~70 μg/l respirable NEFA aerosol (high NEFA group), ~17 μg/l (low NEFA group), or ~70 μg/l FA 6 hrs/day, 5 days/week, for up to 20 months. An identical control group received sham exposures. The NEFA particles of respirable size contained approximately 6% nickel, compared to about 0.3% for FA. Five hamsters/group were sacrificed after 4, 8, 12, or 16 months of exposure. An additional 5 hamsters/group were withdrawn from exposure at the same intervals for observation until 22 months of age when all animals were sacrificed.

To determine pulmonary deposition, translocation and clearance of inhaled fly ash, hamsters received a single 95-minute nose-only exposure to neutron-activated fly ash. The mean respirable aerosol concentration was 470 μg/l. Over a period of 99 days postexposure, the hamsters were sacrificed in groups of six animals. Lungs, liver, kidneys, decapitated and skinned carcass, pelt, head, gastrointestinal tract, urine, and feces were collected for analysis of the radionuclide tracers ^{46}Sc, ^{59}Fe, and ^{60}Co, by γ-ray spectrometry.

Dimethylsulfoxide (DMSO) extracts of NEFA and FA were tested for mutagenicity in the postmitochondrial/*Salmonella* (AMES) assay with and without metabolic activation, using Aroclor-induced hepatic enzyme preparations as the source of activating enzymes. The total concentration of FA

and NEFA in the DMSO solvent was about 50 mg per ml. The concentrations used in the Ames assays of NEFA and FA ranged from 10 to 5000 μg per petri plate.

The final report submitted to the Project Officer also included numerous computer data sheets dealing with: list of observations on individual animals; list of animals with lesions; list of incidence of lesions; and list of incidence by type of tumor. This data is on file in the Health Effects Research Laboratory, 26 W. St. Clair Street, Cincinnati, OH 45268.

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Wellington Moore, Jr. *and* ***William E. Pepelko*** *are the EPA Project Officers (see below).*

The complete report, entitled "Investigation of Effects of Prolonged Inhalation of Nickel-Enriched Fly Ash in Syrian Golden Hamsters," (Order No. PB 81-152 514. Cost: $14.00, subject to change) will be available only from:

National Technical Information Service
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